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(71) Applicant *(for all designated States except US)*: ELI LILLY AND COMPANY [US/US]; Lilly Corporate Center, Indianapolis, IN 46285 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: GRINNELL, Brian, W. [US/US]; 3625 East 71st Street, Indianapolis, IN 46220 (US). HOWEY, Daniel, C. [US/US]; 4524 Lakeridge Drive, Indianapolis, IN 46234 (US). JACKSON, Charles, V. [US/US]; 9921 Leeward Boulevard, Indianapolis, IN 46256 (US).

(74) Agents: CALTRIDER, Steven, P. et al.; Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 (US).

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(54) Title: METHODS FOR TREATING VASCULAR DISORDERS

(57) Abstract

A method of treatment for patients with vascular occlusion and thromboembolic disorders including the acquired disease state of thrombotic stroke, by administering activated protein C. The administration of aPC provides a highly selective therapeutic agent with a low potential for causing bleeding complications. The administration of aPC is beneficial in preventing the local extension of the microvascular and macrovascular occluding arterial thrombus, thereby reducing the neurological deficit resulting from the stroke.

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Title

METHODS FOR TREATING VASCULAR DISORDERS

Field of the Invention

This invention relates to medical science particularly the treatment of vascular disorders with activated protein C.

Background of the Invention

Protein C is a serine protease and naturally occurring anticoagulant that plays a role in the regulation of homeostasis by deactivating Factors V_a and $VIII_a$ in the coagulation cascade. Human protein C is made *in vivo* primarily in the liver as a single polypeptide of 461 amino acids. This precursor molecule undergoes multiple post-translational modifications including 1) cleavage of a 42 amino acid signal sequence; 2) proteolytic removal from the one chain zymogen of the lysine residue at position 155 and the arginine residue at position 156 to make the 2-chain form of the molecule, (i.e., a light chain of 155 amino acid residues attached through a disulfide bridge to the serine protease-containing heavy chain of 262 amino acid residues); 3) vitamin K-dependent carboxylation of nine glutamic acid

residues clustered in the first 42 amino acids of the light chain, resulting in 9 gamma-carboxyglutamic acid residues; and 4) carbohydrate attachment at four sites (one in the light chain and three in the heavy chain). The heavy chain contains the well established serine protease triad of Asp 257, His 211 and Ser 360. Finally, the circulating 2-chain zymogen is activated *in vivo* by thrombin at a phospholipid surface in the presence of calcium ion. Activation results from removal of a dodecapeptide at the N-terminus of the heavy chain, producing activated protein C (aPC) possessing enzymatic activity.

In conjunction with other proteins, protein C functions as perhaps the most important down-regulator of blood coagulation. In other words the protein C enzyme system represents a major physiological mechanism of anticoagulation.

The coagulation system is best viewed as a chain reaction involving the sequential activation of zymogens into active serine proteases eventually producing the enzyme, thrombin, which through limited proteolysis converts plasma fibrinogen into the insoluble gel, fibrin. Two key events in the coagulation cascade are the conversion of clotting factor X to Xa by clotting factor IXa and the conversion of prothrombin into thrombin by clotting factor Xa. Both of these reactions occur on cell surfaces, most notably the platelet surface. Both of these reactions require cofactors. The major cofactors, factors V and VIII, in the system circulate as relatively inactive precursors, but when the first few molecules of thrombin are formed, thrombin loops back and activates the cofactors through limited proteolysis. The activated cofactors, Va and VIIIa, accelerate both the conversion of prothrombin into thrombin and also the conversion of factor X to factor Xa by approximately five orders of magnitude. Activated protein C overwhelmingly prefers two plasma protein substrates which it hydrolyzes and irreversibly destroys. These plasma protein substrates are the activated forms of the clotting

cofactors, Va and VIIIa. Activated protein C only minimally degrades the inactive precursors, clotting factors V and VIII. Activated protein C in dogs has been shown to sharply increase circulating levels of the major physiological fibrinolytic enzyme, tissue plasminogen activator (tPA). Activated protein C has been shown *in vitro* to enhance the lysis of fibrin in human whole blood. Therefore, activated protein C represents an important adjunct to *in vivo* fibrinolysis in man.

Today, there are few effective treatments available for vascular occlusions, including thrombotic stroke. Treatment with tPA, if administered within three hours from the onset of the stroke, has been recently approved by the FDA. Treatment of strokes with either heparin or oral anticoagulants, although occasionally beneficial, carries a high risk for bleeding into the infarcted brain area.

The use of recombinant aPC (r-aPC) in the treatment of thrombotic occlusion or thromboembolism in a baboon model has been presented by Griffin, *et al.* in U.S. Patent No.5,084,274. Griffin claimed dose levels in the range of 0.2 mg/kg/hr to 1.1 mg/kg/hr for the treatment of thrombotic occlusion. However, applicants have found that these dose levels are in a range significantly above the toxicological level of r-aPC. For example, pre-clinical toxicology studies in non-human primates indicate the safety of r-aPC for a 96 hour infusion is limited at a top dose of around 0.05 mg/kg/hr. Therefore, the lowest dose level taught by Griffin, *et al.*, i.e. 0.2 mg/kg/hr, is at a level 4 times greater than the toxic dose established by applicants for humans. Thus, even the lowest dose level taught by Griffin would carry a high risk for bleeding into the infarcted brain area, thereby aggravating the neurological deficit accompanying the stroke. Accordingly, even in view of the teaching of Griffin, *et al.*, there remains a need to identify an effective therapy of arterial thrombus formation in humans with aPC.

Contrary to the teachings of prior investigators, applicants have discovered that only low dose therapy with r-aPC is useful in the treatment of thrombotic stroke. The administration of aPC is also beneficial in preventing the local extension of the microvascular and macrovascular occluding arterial thrombus, thereby reducing the neurological deficit resulting from the stroke.

Summary of the Invention

The present invention provides a method of treatment for human patients with vascular occlusive and arterial thromboembolic disorders which comprises administering to said patient a dosage of about 0.01mg/kg/hr to about 0.05mg/kg/hr of activated protein C.

This invention also provides a unit dosage form suitable for administration by continuous infusion which comprises a unit dosage receptacle containing about 5 mg to about 20mg of activated protein C suitable for administering a dosage of about 0.01mg/kg/hr to about 0.05mg/kg/hr.

Detailed Description of the Invention

For purposes of the present invention, as disclosed and claimed herein, the following terms are as defined below.

aPC or activated protein C refers to protein C whether recombinant or plasma derived. aPC includes and is preferably human protein C although aPC may also include other species or derivatives having protein C proteolytic, amidolytic, esterolytic, and biological (anticoagulant or pro-fibrinolytic) activities. Examples of protein C derivatives are described by Gerlitz, *et al.*, U.S. patent No. 5,453,373, and Foster, *et al.*, U.S. patent No. 5,516,650, the entire teachings of which are hereby included by reference.

APTT - activated partial thromboplastin time.

AU - amidolytic units.

HPC - human protein C zymogen.

MEA - 2-aminoethanol.

tPA - tissue plasminogen activator

r-HPC - recombinant human protein C zymogen.

r-aPC - recombinant activated protein C produced by activating protein C zymogen *in vitro* or *in vivo* or by direct secretion of the activated form of protein C from procaryotic cells, eukaryotic cells, or transgenic animals including, for example, secretion from human kidney 293 cells as a zymogen then purified and activated by techniques well known to the skilled artisan and demonstrated in Yan, U.S. Patent No. 4,981,952, and Cottingham, WO 97/20043, the entire teachings of which are herein incorporated by reference.

Continuous infusion - continuing substantially uninterrupted the introduction of a solution into a blood vessel for a specified period of time.

Bolus injection - the injection of a drug in a defined quantity (called a bolus) over a period of time up to about 120 minutes.

Suitable for administration - A formulation or solution preferably prepared from lyophilized aPC that is appropriate to be given as a therapeutic agent.

Zymogen - an enzymatically inactive precursor of a proteolytic enzyme. Protein C zymogen, as used herein, refers to secreted, inactive forms, whether one chain or two chains, of protein C.

Applicants have found that pre-clinical toxicology studies in non-human primates indicate the safety of r-aPC for a 96 hour infusion is limited at a top dose of around 0.05 mg/kg/hr. These data are unexpected when compared to the prior art. In fact, the dose levels of r-aPC for humans that have been based on previous pre-clinical and clinical studies are above the toxicological range established in the above toxicological studies.

The present invention provides a method of treatment for human patients with vascular occlusive and arterial thromboembolic disorders which comprises administering to

said patient a dosage of about 0.01 mg/kg/hr to about 0.05 mg/kg/hr of activated protein C. Administering activated protein C at low dose levels is useful for the treatment of thrombotic stroke without the concomitant bleeding problems that may be associated with high dose levels. The present invention further demonstrates using recombinant human protein C (r-aPC) in a human clinical trial to determine blood plasma concentration of r-aPC (Example 1).

The present invention also demonstrates the effect of intravenous administration of r-aPC on reperfusion of totally occluded coronary arteries in a canine model of occlusive coronary artery thrombosis (Example 2). Surprisingly, five of six animals treated with r-aPC demonstrated vessel reperfusion compared to vessel reperfusion in none of the six control animals.

The present invention relates to treatment with activated protein C of vascular occlusive or arterial thromboembolic disorders, including thrombotic stroke, peripheral arterial thrombosis, emboli originating from the heart or peripheral arteries, acute myocardial infarction, and coronary arterial disease.

The aPC can be formulated according to known methods to prepare pharmaceutically useful compositions. The aPC is preferably administered parenterally to ensure its delivery into the bloodstream in an effective form by injecting the appropriate dose as continuous infusion for about one to about forty eight hours. More preferably, the appropriate dose of aPC will be administered by continuous infusion for about 4 to about 36 hours. Even more preferably, the appropriate dose of aPC will be administered by continuous infusion for about 12 to about 24 hours. Most preferably, the appropriate dose of aPC will be administered by continuous infusion for about 24 hours. The administration

of aPC will begin as soon as possible following diagnosis of the stroke.

The amount of aPC administered is from about 0.01 mg/kg/hr to about 0.05 mg/kg/hr which is equivalent to about 20 mg/70 kg/24 hours to about 84 mg/70 kg/24 hours. While the dose level is identified as a specific amount per 24 hours, one skilled in the art would recognize that this is a designation of the dose level and is not necessarily limited to a 24 hour infusion but may include continuous infusion for various times, for example, from about one hour to about forty eight hours. More preferably the amount of aPC administered is about 0.01 mg/kg/hr to about 0.04 mg/kg/hr (about 20 mg/70 kg/24 hours to about 67 mg/70 kg/24 hours). While more preferably the amount of aPC administered will be about 0.01 mg/kg/hr to about 0.03 mg/kg/hr (about 20 mg/70 kg/24 hours to about 50 mg/70 kg/24 hours). The most preferable amount of aPC administered is about 0.024 mg/kg/hr (about 40 mg/70 kg/24 hours).

Alternatively, the aPC will be administered by injecting a portion of the appropriate dose per hour as a bolus injection over a time from about 5 minutes to about 30 minutes, followed by continuous infusion of the appropriate dose for about twenty three hours to about 47 hours which results in the appropriate dose administered over 24 hours to 48 hours.

As noted previously, the dosage levels of aPC presented above are in contrast to those presented by Griffin, *et al.* Griffin claimed dose levels in the range of 0.2 mg/kg/hr to 1.1 mg/kg/hr for the treatment of thrombotic occlusion. In contrast, the dose levels claimed herein are equivalent to a tenth of this dose or a range of about 0.01mg/kg/hr to about 0.05mg/kg/hr. The most preferable dose level of aPC to be administered for thrombolitic occlusion as described herein will be about 0.024mg/kg/hr. It is significant to note that the most preferable dose level of 0.024mg/kg/hr as indicated herein is 8 fold less than the lowest dose level claimed by

Griffin and 44 fold less than the highest dose level claimed by Griffin.

Preparation 1

Preparation of Human Protein C

Recombinant human protein C (rHPC) was produced in Human Kidney 293 cells by techniques well known to the skilled artisan such as those set forth in Yan, U.S. Patent No. 4,981,952, the entire teaching of which is herein incorporated by reference. The gene encoding human protein C is disclosed and claimed in Bang, *et al.*, U.S. Patent No. 4,775,624, the entire teaching of which is incorporated herein by reference. The plasmid used to express human protein C in 293 cells was plasmid pLPC which is disclosed in Bang, *et al.*, U.S. Patent No. 4,992,373, the entire teaching of which is incorporated herein by reference. The construction of plasmid pLPC is also described in European Patent Publication No. 0 445 939, and in Grinnell, *et al.*, 1987, Bio/Technology 5:1189-1192, the teachings of which are also incorporated herein by reference. Briefly, the plasmid was transfected into 293 cells, then stable transformants were identified, subcultured and grown in serum-free media. After fermentation, cell-free medium was obtained by microfiltration.

The human protein C was separated from the culture fluid by an adaptation of the techniques of Yan, U.S. Patent No. 4,981,952, the entire teaching of which is herein incorporated by reference. The clarified medium was made 4 mM in EDTA before it was absorbed to an anion exchange resin (Fast-Flow Q, Pharmacia). After washing with 4 column volumes of 20 mM Tris, 200 mM NaCl, pH 7.4 and 2 column volumes of 20 mM Tris, 150 mM NaCl, pH 7.4, the bound recombinant human protein C zymogen was eluted with 20 mM Tris, 150 mM NaCl, 10 mM $CaCl_2$, pH 7.4. The eluted protein was greater than 95% pure after elution as judged by SDS-polyacrylamide gel electrophoresis.

Further purification of the protein was accomplished by making the protein 3 M in NaCl followed by adsorption to a hydrophobic interaction resin (Toyopearl Phenyl 650M, TosoHaas) equilibrated in 20 mM Tris, 3 M NaCl, 10 mM $CaCl_2$, pH 7.4. After washing with 2 column volumes of equilibration buffer without $CaCl_2$, the recombinant human protein C was eluted with 20 mM Tris, pH 7.4.

The eluted protein was prepared for activation by removal of residual calcium. The recombinant human protein C was passed over a metal affinity column (Chelex-100, Bio-Rad) to remove calcium and again bound to an anion exchanger (Fast Flow Q, Pharmacia). Both of these columns were arranged in series and equilibrated in 20 mM Tris, 150 mM NaCl, 5 mM EDTA, pH 6.5. Following loading of the protein, the Chelex-100 column was washed with one column volume of the same buffer before disconnecting it from the series. The anion exchange column was washed with 3 column volumes of equilibration buffer before eluting the protein with 0.4 M NaCl, 20 mM Tris-acetate, pH 6.5. Protein concentrations of recombinant human protein C and recombinant activated protein C solutions were measured by UV 280 nm extinction $E^{0.1\%}=1.85$ or 1.95, respectively.

Preparation 2

Activation of Recombinant Human Protein C

Bovine thrombin was coupled to Activated CH-Sepharose 4B (Pharmacia) in the presence of 50 mM HEPES, pH 7.5 at 4°C. The coupling reaction was done on resin already packed into a column using approximately 5000 units thrombin/ml resin. The thrombin solution was circulated through the column for approximately 3 hours before adding MEA to a concentration of 0.6 ml/l of circulating solution. The MEA-containing solution was circulated for an additional 10-12 hours to assure complete blockage of the unreacted amines on the resin. Following blocking, the thrombin-coupled resin was washed with 10 column volumes of 1 M NaCl, 20 mM Tris,

pH 6.5 to remove all non-specifically bound protein, and was used in activation reactions after equilibrating in activation buffer.

Purified rHPC was made 5mM in EDTA (to chelate any residual calcium) and diluted to a concentration of 2 mg/ml with 20 mM Tris, pH 7.4 or 20 mM Tris-acetate, pH 6.5. This material was passed through a thrombin column equilibrated at 37°C with 50 mM NaCl and either 20 mM Tris pH 7.4 or 20 mM Tris-acetate pH 6.5. The flow rate was adjusted to allow for approximately 20 min. of contact time between the rHPC and thrombin resin. The effluent was collected and immediately assayed for amidolytic activity. If the material did not have a specific activity (amidolytic) comparable to an established standard of aPC, it was recycled over the thrombin column to activate the rHPC to completion. This was followed by 1:1 dilution of the material with 20 mM buffer as above, with a pH of anywhere between 7.4 or 6.0 (lower pH being preferable to prevent autodegradation) to keep the aPC at lower concentrations while it awaited the next processing step.

Removal of leached thrombin from the aPC material was accomplished by binding the aPC to an anion exchange resin (Fast Flow Q, Pharmacia) equilibrated in activation buffer (either 20 mM Tris, pH 7.4 or preferably 20 mM Tris-acetate, pH 6.5) with 150 mM NaCl. Thrombin passes through the column and elutes during a 2-6 column volume wash with 20 mM equilibration buffer. Bound aPC is eluted with a step gradient using 0.4 M NaCl in either 5 mM Tris-acetate, pH 6.5 or 20 mM Tris, pH 7.4. Higher volume washes of the column facilitated more complete removal of the dodecapeptide. The material eluted from this column was stored either in a frozen solution (-20°C) or as a lyophilized powder.

The amidolytic activity (AU) of aPC was determined by release of p-nitroanaline from the synthetic substrate H-D-Phe-Pip-Arg-p-nitroanilide (S-2238) purchased from Kabi Vitrum using a Beckman DU-7400 diode array

spectrophotometer. One unit of activated protein C was defined as the amount of enzyme required for the release of 1 μmol of p-nitroaniline in 1 min. at 25°C, pH 7.4, using an extinction coefficient for p-nitroaniline at 405 nm of 9620 $M^{-1}cm^{-1}$.

The anticoagulant activity of activated protein C was determined by measuring the prolongation of the clotting time in the activated partial thromboplastin time (APTT) clotting assay. A standard curve was prepared in dilution buffer (1 mg/ml radioimmunoassay grade BSA, 20 mM Tris, pH 7.4, 150 mM NaCl, 0.02% NaN_3) ranging in protein C concentration from 125-1000 ng/ml, while samples were prepared at several dilutions in this concentration range. To each sample cuvette, 50 μl of cold horse plasma and 50 μl of reconstituted activated partial thromboplastin time reagent (APTT Reagent, Sigma) were added and incubated at 37 °C for 5 min. After incubation, 50 μl of the appropriate samples or standards were added to each cuvette. Dilution buffer was used in place of sample or standard to determine basal clotting time. The timer of the fibrometer (CoA Screener Hemostasis Analyzer, American Labor) was started upon the addition of 50 μl 37°C 30 mM $CaCl_2$ to each sample or standard. Activated protein C concentration in samples are calculated from the linear regression equation of the standard curve. Clotting times reported here are the average of a minimum of three replicates, including standard curve samples.

The above descriptions enable one with appropriate skill in the art to prepare aPC for utilization it in the treatment of thrombotic stroke.

Example 1

Human Plasma Levels of aPC

Six human patients received an i.v. infusion of aPC at 1 mg/m^2 /hour or about 0.024 mg/kg/hr over a 24 hour period. The aPC administered was a lyophilized formulation

containing 10 mg aPC, 5 mM Tris acetate buffer and 100 mM sodium chloride reconstituted with two ml of water and adjusted to pH 6.5.

Plasma concentrations of aPC were measured using an Immunocapture-Amidolytic Assay. Blood was collected in the presence of citrate anticoagulant and benzamidine, a reversible inhibitor of aPC. The enzyme was captured from plasma by an aPC specific murine monoclonal antibody, C3, immobilized on a microtiter plate. The inhibitor was removed by washing and the amidolytic activity or aPC was measured using an oligopeptide chromogenic substrate. Following incubation for 16-20 h at 37° C, the absorbance was measured at 405 nm and data are analyzed by a weighted linear curve-fitting algorithm. aPC concentrations were estimated from a standard curve ranging in concentrations from 0-100 ng/ml. The limit of quantitation of the assay was 1.0 ng/ml. The aPC dose levels and plasma concentrations were measured at about 24 hours. The plasma ranges are from 2 ng/ml to less than 100 ng/ml. The preferred plasma ranges are from about 20 ng/ml to 80 ng/ml. Most preferably plasma ranges are from about 30 ng/ml to about 60 ng/ml and still more preferably about 50 ng/ml. Thus, the dose of 0.024 mg/kg/hr yields the most preferable plasma concentration of 50 ng/ml at 24 hours for

treatment of thrombotic stroke without the concomitant bleeding problems from higher dose levels.

Example 2

Induced Reperfusion in a Canine Model of Occlusive Coronary Artery Thrombosis

Twelve dogs (17-22 kg, either sex, Butler Farms) were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and ventilated with room air. Cannulas were placed for measurement of blood pressure, drug administration and blood sampling in the carotid artery, femoral vein, and jugular vein; respectively. A left thoracotomy was performed, the heart was suspended in a pericardial cradle and a 2 cm segment of the left circumflex coronary artery (LCCA) was isolated proximal to the first main diagonal branch. The LCCA was instrumented with an electromagnetic flow probe, stimulating electrode, and an external occluder to measure coronary blood flow, produce vessel injury, and provide critical stenosis; respectively. Vessel injury was caused by placing the stimulating electrode (anode) in contact with the intimal side of the vessel and stimulating the anode with 100 μ A d.c. current (the circuit was completed by placing the cathode in a subcutaneous site). The injury current was continued for 60 minutes and then stopped whether the vessel has occluded or not. Vessels reached total occlusion in approximately 60 minutes from the initiation of vessel injury. Thirty minutes after total vessel occlusion (established as zero coronary blood flow for 30 minutes) a continuous intravenous infusion of 2.0 mg/kg/hr aPC or 20 ml TRIS buffer, pH 7.4 (vehicle group) was infused for 2 hr. The preparations were followed for 4 hrs beginning from the point of initiation of LCCA injury. Arterial blood pressure, heart rate and coronary blood flow were acquired and analyzed. At different time points throughout the experiment, blood samples were drawn to determine whole blood clotting times (Hemochron 801), and gingival template bleeding times were determined using a

Simplate II bleeding time device. A second set of blood samples (citrated) were collected throughout the experiment for determination of plasma plasminogen activator inhibitor-1 (PAI-1). Plasma PAI-1 levels were determined using an IMUBIND ™ plasma PAI-1 ELISA kit (American Diagnostica). All data (reported as mean ± SEM) were analyzed for statistical differences using single ANOVA followed by Student-Neuman-Keuls analysis for significance at the level $p<.05$. Incidence of reperfusion and patency were analyzed using Fisher's Exact test at a level of $p<.05$.

A continuous infusion of 2.0 mg/kg/hr aPC produced a 6 fold increase in APTT whole blood clotting time by the end of the 2 hr drug infusion (table 1). APTT had begun to return to normal values by the end of the experiment. There was no observable effect on thrombin clotting time or template bleeding time. Results are set forth in Table 2.

Table 2

Effects of aPC on Coagulation and Template Bleeding Times in the Anesthetized Dog

Treatment	Parameter	Predrug	60 min Inf.	120 min Inf.	End
Vehicle [a] (n=6)	Thrombin Time (sec)	36±1	38±4	33±1	34±1
	APTT(sec)	100±6	95±5	89±10	91±10
	Template Bleeding Time (sec)	132±15	182±14	152±15	159±13
aPC* (n=6)	Thrombin Time (sec)	33±1	34±1	34±1	34±1
	APTT (sec)	96±6	573±237	670±209 *	138±13 *
	Template Bleeding Time (sec)	199±41	272±84	204±20	193±39

The dosing regimen used for the Vehicle group was 20 ml of TRIS-Buffered Saline infused for 2 hr. and aPC (2.0 mg/kg/hr x 2h) administration began 30 minutes after total vessel occlusion.

* Denotes a statistical difference at the level $p<.05$ versus the vehicle group. Each value represents the mean±SEM.

Table 3 illustrates the effects of intravenous administration of aPC on reperfusion of totally occluded coronary arteries. Time to total thrombotic occlusion of the coronary arteries was similar between the 2 groups; 66±7 and 62±6 minutes, vehicle-treated and aPC-treated, respectively. Five of six vessels in the aPC-treated group demonstrated reperfusion compared to none of the 6 vessels receiving vehicle; time to reperfusion in the aPC-treated group was 128±17 min. Coronary blood flow in the aPC treated group was significantly greater than the corresponding vehicle-treated group; the aPC-treated group reached 13.7±2.7 ml/min during the reperfusion period and a flow volume of 1069±623 ml (this represents a restoration of approximately 60-70% of the pre-thrombosis coronary blood flow in this group). Three of the 5 vessels exposed to aPC were still patent at the end of the 4 hr experiment. Thus, the data demonstrates that aPC is effective in the treatment of occlusive coronary artery thrombosis in a canine model.

Table 3

Effects of aPC on Restoration of Coronary Blood Flow in the Canine Coronary Artery Thrombosis Model

Parameter	Vehicle (n=6)	aPC (n=6)
Time to Occlusion (min)	66±7	62±6
Thrombus Mass (mg)	10.8±2.1	8.2±1.2
Incidence of Reperfusion	0	5 of 6 *
Time to Reperfusion (min)	0	128±17 *
Vessel Patency @ End of Experiment	0 of 6	3 of 5
CBF during Reperfusion (ml/min)	0	13.7±2.7 *
Reperfusion Volume (ml)	0	1069±623

* Denotes a statistical difference at the level $p<.05$ versus the vehicle group. Each value represents the mean±SEM.

Blood samples drawn throughout the each experiment demonstrated that there was a significant correlation with the intravenous infusion of aPC and circulating levels of plasminogen activator inhibitor-1 (PAI-1). By the end of the intravenous infusion of aPC, plasma PAI-1 levels had decreased by 80%. Upon cessation of the infusion of aPC, plasma PAI-1 levels began to return to pre-infusion levels.

Although these dosage levels in this canine model appear to be higher than the claimed dosage levels for humans, Applicants have found that the dog is especially insensitive to human activated protein C, therefore the claimed dosage levels are appropriate for humans.

What is claimed is:

1. A method of treatment for human patients with vascular occlusive and arterial thromboembolic disorders, which comprises administering to said patient a dosage of about 0.01mg/kg/hr to about 0.05mg/kg/hr of activated protein C.

2. The method of Claim 1 wherein the vascular occlusive or thromboembolic disorder is thrombotic stroke.

3. The method according to Claim 2 which comprises administering to said patient about 0.01mg/kg/hr to about 0.03mg/kg/hr activated protein C.

4. The method according to Claim 3 which comprises administering to said patient about 0.024mg/kg/hr activated protein C.

5. The method according to Claim 4 wherein the activated protein C is human activated protein C.

6. A method of Claim 1 wherein the activated protein C is administered in a continuous infusion for about 1 hour to about 48 hours.

7. A method of Claim 6 wherein the activated protein C is administered in a continuous infusion for about 12 hours to about 36 hours.

8. A method of Claim 7 wherein the activated protein C is administered in a continuous infusion for about 24 hours.

9. The method according to Claim 8 wherein the activated protein C is human activated protein C.

10. The method according to Claim 9 which comprises administering about 0.024mg/kg/hr activated protein C.

11. The method of Claim 1 wherein the activated protein C is administered in a bolus injection.

12. The method of Claim 11 wherein the bolus injection of activated protein C is followed by a continuous infusion.

13. A unit dosage receptacle containing from 5 to 20 mg of activated protein C.

14. A receptacle as claimed in Claim 13, in which the activated protein C is lyophilized.

15. A receptacle as claimed in Claim 13, in which the activated protein C is dissolved in a sterile solution suitable for administration at a dosage of from 0.01 mg/kg/hr to 0.05 mg/kg/hr.

16. A unit dosage of Claim 13, wherein the administration is by continuous infusion for about 1 to about 48 hours.

17. Activated protein C for use as a medicament for the treatment of vascular occlusive and arterial thromboembolic disorders at a dose of about 0.01mg/kg/hr to about 0.05mg/kg/hr.